The Thesis Committee for Allison Mooney Griffith
Certifies that this is the approved version of the following thesis:

Identifying Functions of Down Syndrome-Related Genes Using RNA Interference in *C. elegans*

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Jon Pierce-Shimomura

Edward Marcotte

Identifying Functions of Down Syndrome-Related Genes Using RNA Interference in *C. elegans*

by

Allison Mooney Griffith, B.S.

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Abstract

Identifying Functions of Down Syndrome-Related Genes Using RNA Interference in *C. elegans*

Allison Mooney Griffith, M.A.

The University of Texas at Austin, 2010

Supervisor: Jon Pierce-Shimomura

Down syndrome is one of the most common genetic disorders, resulting in a range of neurological and neuromuscular disabilities. Although the presence of specific disabilities varies among individuals with Down syndrome, all individuals with Down syndrome are born with hypotonia (low muscle tone) and over half with congenital heart defects. Later in life, all individuals demonstrate intellectual disabilities to varying degrees, while many also develop early-onset Alzheimer's disease. While the cause of Down syndrome is known to be a triplication of the 21st chromosome, it is unknown how this extraneous genetic material causes the development of these phenotypes. We have begun research into the biological basis of these disabilities using the tiny nematode, *Caenorhabditis elegans* as a genetic model. We used the technique RNA interference

(RNAi), which allows us to study the *in vivo* function of genes by knocking down their expression one at a time in a living, behaving animal. We have used this technique to systematically study the *in vivo* function for genes involved in Down syndrome. To this end, we identified and knocked down *C. elegans* genes with sequence similarity to 67% of genes on the human 21st chromosome genes. We used these RNAi-treated worms to investigate the neuromuscular function of human chromosome 21 gene equivalents by assaying locomotion and pharyngeal pumping in a blinded screen. We used locomotion as a measure of neurological and neuromuscular function, while we used pharyngeal pumping as a model for cardiac function. We also performed an aldicarb screen to examine the role of some of these genes in the function of the synapse. Our experiments have provided valuable insight into the *in vivo* function of the vast majority of genes on the human 21st chromosome. This will be vital to identify genes that are potentially involved in eliciting Down syndrome-related phenotypes, laying the groundwork for further studies into the neurobiology of Down syndrome.

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Chapter 1: Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability, occurring at a rate of 1 in ~700 live births in the United States (Canfield *et al.*, 2006). People with DS experience a wide variety of neurological and neuromuscular symptoms in addition to varying degrees of intellectual disability and learning and memory problems (Korenberg *et al*. 1994). For example, DS is the leading known cause of neonatal heart defects, leading to a high infant mortality rate without surgical intervention (Korenberg *et al*. 1994; Vis *et al.* 2009). Another common symptom of DS, muscle weakness (hypotonia), causes problems with both gross and fine motor skills, which leads those affected to have trouble speaking, writing, and walking (Pitetti *et al*. 1992). Furthermore, nearly everyone with DS develops Alzheimer's-like dementia once they reach middle age (Coyle *et al*. 1988). By studying the genetic origin of these common neurological and neuromuscular phenotypes, we may be able to develop treatments or therapies to help improve the quality of life for people with DS.

While the direct causes of these phenotypes are unknown, the biology of DS has been studied to an extent. For example, we have had evidence for over 50 years that DS is caused by trisomy 21, the triplication of the human somatic autosome 21 (HSA21) (Jacobs *et al*. 1959). However, the mechanisms by which HSA21 causes the DS-associated phenotypes are unknown. One of the first models explaining the link between DS and trisomy 21 suggested that the burden of the extra genetic material put strain on cellular processes on the DS patient, causing the manifestation of all DS symptoms (Patterson 2009). However, research on individuals with partial trisomy 21 has shown

that the amount of extraneous genetic material does not account for all the symptoms associated with DS (Korenberg *et al.* 1994; Lyle *et al*. 2009). A more recent theory suggests that a group of about 30 HSA21 genes, known collectively as the Down Syndrome Critical Region (DSCR), are responsible for all DS-associated phenotypes (Rahmani *et al*. 1989). A mouse model with trisomy of DSCR-homolog genes has been developed (Ts1Rhr) (Olson *et al*. 2004). Observations of these mice have accounted for only some phenotypes, suggesting that the DSCR is not solely responsible for the whole complement of DS-associated phenotypes (Belichenko *et al.* 2009; Olson *et al*. 2004). A new mouse model of DS, trisomic for over 75% of all HSA21 orthologs, exhibits more DS-type phenotypes than the Ts1Rhr model (Yu *et al*. 2010). This led to the current theory that each DS phenotype is caused by a single, separate gene or small group of genes on HSA21 (Lyle *et al*. 2009). In other words, the heart defects may be caused by a single gene or group of genes, while hypotonia may be caused by a second gene or group of genes, and so on.

Few single genes have been tested for specific genotype-phenotype correlations. For example, overexpression of the *Drosophila* homolog of *SYNJ*, a HSA21 gene, was shown to result in synaptic signaling and locomotion abnormalities in flies (Chang and Min 2009). Furthermore, a study of another HSA21 gene, *Dyrk1A*, has shown that overexpression in mouse models results in DS-like gene splicing errors in neurons (Toiber *et al*. 2010).

To identify candidate genes that contribute to specific defects, it would be useful to know the normal *in vivo* function of all HSA21 genes. Presumably, specific defects in

cardiac function will be more likely to be caused by genes that normally function in the heart, while specific neurological defects will be more likely to be caused by genes that function in the nervous system. While the list of protein coding genes on HSA21 has been compiled through the human genome project, a comprehensive list of the *in vivo* functions of these genes is mostly incomplete. Our project begins to fill this critical gap in knowledge by studying the function of HSA21 gene equivalents in a genetically tractable organism, the tiny nematode *Caneorhabditis elegans.*

Unfortunately, human studies into the genetic causes of DS-related phenotypes are minimal, with most research focused on case studies and basic observations of the physiology of people with DS. However, work studying people with partial trisomy 21 has described several 'susceptibility regions' on HSA21 that are correlated with various DS-associated phenotypes (Lyle *et al*. 2009). The incidence of partial trisomy 21 in humans is exceptionally low, causing these studies to progress slowly and existing data to be plagued by low sample numbers and inconsistent phenotypic observations, making statistical analyses questionable.

About half of the genes on HSA21 are completely unstudied, making it impossible to know if they have a function in the generation of DS-related phenotypes. With the exception of the few studies mentioned above, almost all research into the biological basis of DS has been performed in mouse models, focusing on the gross effects of large groups of genes (Patterson 2009). Ultimately, an overexpression study will be vital to examine the effects of increasing the copy number of each gene. However, it is

impractical to begin such a study without knowing the function of each gene we propose to study.

The goals of this project are two-fold. First, we will begin to determine the general *in vivo* function of all HSA21 genes by studying their evolutionarily conserved equivalents in *C. elegans*. Secondly, we plan to identify those genes which are involved in the function of the synapse. This knowledge will allow us to predict which genes likely contribute the neurological and neuromuscular phenotypes associated with DS.

C. elegans is an exceptional model for genetic studies. It is a well-established genetic model for human disease, exhibiting sequence similarity with at least 42% of human disease-causing genes (Culetto and Sattelle 2000). Worms are easy to raise and grow quickly, allowing large-scale studies to be performed with minimal investments of time and money (Timmons *et al*. 2001).

To identify the function of HSA21 gene equivalents in *C. elegans*, we used a technique that will let us observe *in vivo* effects of gene knockdown. RNA interference (RNAi) allows us to observe gene-specific knockdown effects in a living, behaving worm without requiring the generation of mutant strains (Fire *et al.* 1998). Feeding worms with bacteria expressing double stranded RNA whose sequence is designed to match that of a gene of interest will quickly and easily knock down the expression of that gene in the worm (Fire *et al*. 1998; Timmons *et al*. 2001). Large-scale RNAi screens are simple to do in *C. elegans*, allowing us to quickly examine the behavioral effects of hundreds of genes.

Because the *C. elegans* nervous system is very compact, with only 302 neurons connected by only ~8,000 synapses, we can observe a worm's locomotion and other behaviors to assess neurological function. With only 120 motor neurons, an abnormality in any one neuron will likely manifest as an abnormal gait (Chen *et al.* 2006). Specific changes in locomotion have been shown to indicate underlying abnormal neural function (Pierce-Shimomura *et al*. 2008). For example, defects in GABA neurons cause the worm to contract abnormally when mechanically stimulated on the head (Cinar *et al.* 2005).

In addition to identifying genes involved in locomotion and neuromuscular functions, we can examine other pathologies associated with DS. For example, DS is the most common known cause of congenital heart disease. We can use the worm to model this pathology as well. The pharynx, a muscular feeding organ with physiological and molecular similarities to the human heart, is an excellent model for cardiac function (Avery and Horvitz 1989; Avery and Shtonda 2003; Haun *et al*. 1998). Observations of the pharynx pumping and morphology can allow us to identify genes that may be involved in the congenital cardiac defects associated with DS. Therefore, through behavioral observation of RNAi-treated worms, we can make inferences about the function of HSA21 genes.

As discussed above, RNAi screening allows us to target single, specific genes, reducing the incidence of off-target effects that can occur with the use of mutagens or pre-existing mutant *C. elegans* strains. The procedure we used (as described in Chapter 3) is a simple way to examine the effects of many genes in a short period of time. We decided to use RNAi instead of simply examining existing strains for three main reasons.

First, our RNAi library covers more genes than are available as mutants. Second, all *C. elegans* mutant strains contain secondary mutations in other genes, which can often cause errors when attempting to assign a function to single genes. RNAi in the worm is a simple assay with which to examine the function of HSA21 genes in an unbiased way. Third, deletion of about 20% of genes in *C. elegans* produces lethality, making it inconvenient to study their *in vivo* function. By contrast, RNAi techniques allow us to study the function of these critical genes by knocking down their expression levels after embryonic development, or by knocking down expression to sub-lethal levels.

Functional synapses are vital for proper neurological and neuromuscular function. To identify genes that may be involved in the function of the synapse we used an aldicarb sensitivity assay (Brenner, 1974). Aldicarb is an acetylcholinesterase inhibitor which induces paralysis in worms by increasing the concentration of acetylcholine at the neuromuscular synapses. By observing RNAi-treated worms' responses to aldicarb, we can learn about the gene's mechanism of function.

Chapter 2: Generation of list of HSA21 gene equivalents in *C. elegans*

Purpose

To ensure that we focused on genes relevant to DS, identification of *C. elegans* gene equivalents of HSA21 genes was necessary before beginning our screen. We chose to examine the protein-coding genes of HSA21 because it is likely that the phenotypes associated with DS are due to an over-expression of these genes. People with DS exhibit abnormally high concentrations of proteins encoded by the 21st chromosome, often 1.5 times higher than normal (Lockstone *et al*. 2007). The relatively small number of protein-coding genes (226) on HSA21 makes this a project that could be completed in a reasonable period of time. Like humans, *C. elegans* has over 20,000 genes. More than half of these genes have sequence similarity to human genes (*C. elegans* Sequencing Consortium, 1998). Therefore, it was likely that we would find many gene equivalents between HSA21 genes and *C. elegans*.

Methods

We began by collecting sequences of HSA21 protein-coding genes predicted by the manually curated Vertebrate Genome Annotation genome database (VEGA, July 2009, Wilming *et al.* 2007). VEGA identified 226 genes on HSA21 as protein-coding through sequence analysis. We then used these gene sequences to BlastP against the *C. elegans* genome (Wormbase Release WS203). Worm genes showing good sequence similarity (e value>1E-4) were considered orthologs. The remaining genes were then searched for paralog relationships to *C. elegans* genes (O'Brien *et al*. 2005). Paralogs are genes that have diverged enough to prevent standard sequence identification but are still

functionally similar (O'Brien *et al*. 2005). Together, we will call the orthologs and paralogs 'gene equivalents.'

Results

Two general groups of human genes were identified, those with *C. elegans* gene equivalents and those without (Figure 1). The majority of the human protein-coding genes (154) were represented by 192 *C. elegans* genes. This means that 67% of HSA21 genes are represented in the worm. Most of these genes (119) were found to have orthologs in the *C. elegans* genome, while a few were identified as paralogs (35).

The remainder of the HSA21 genes (72) are unrepresented in the *C. elegans* genome. A large expansion of keratin genes accounts for 46 of the genes on HSA21. Keratin is involved in human hair and skin growth, and thus is not present in *C. elegans*. Interestingly, people with DS often have atypical hair and skin morphology. For example, alopecia (baldness) is common in this population (Korenberg 1994). Although keratin is over-expressed in people with DS, it is unlikely that it is involved in neurological disabilities associated with the disease.

Beside the keratin genes on HSA21, an additional set of 26 genes had no equivalent *C. elegans* gene ('Unique Genes' in Figure 1). These genes all have vertebrate- or mammalian-specific functions, and therefore are not present in the *C. elegans* genome. For example, the genes *IFNGR2* and *MX1* are involved in vertebrate-specific immune functions. One gene in this group which may have a role in neurological function is *OLIG1*. It is involved in the function of oligodendrocytes, which are involved in the formation of the myelin sheath in higher vertebrates (Ligon *et al*.

2006, Wu *et al*. 2006). It is possible that myelination could be involved in the abnormal neurological function of people with DS. However, the invertebrate *C. elegans* does not have myelin, and no *OLIG1* gene equivalent is present in the worm genome.

Discussion

Based on the sequence similarities between human and *C. elegans* genes, we conclude that there is sufficient genetic identity to warrant the use of *C. elegans* as a model to study the function of HSA21 genes. A significant portion of HSA21 has sequence similarity to the *C. elegans* genes (67% of all HSA21 genes are represented in the *C. elegans* genome, 85% when keratin genes are excluded). This means that most of the genes involved in the neurological and neuromuscular abnormalities associated with DS are likely represented in *C. elegans*.

Chapter 3: Primary RNAi screen of HSA21 equivalents

Purpose

Even though DS is a disease of overexpression, simple techniques to knock down gene expression can reveal a lot about the genetic origin for DS-associated phenotypes. RNA interference (RNAi) has proven its usefulness as a method of studying gene function and human diseases including Alzheimer's disease, metabolic syndrome and cancer (reviewed by Wolters and MacKeigan 2008). RNAi can easily be applied to *C. elegans* to quickly observe the effects of gene knockdown in a living, behaving animal. By feeding worms bacteria expressing RNA with sequence similarity to the gene equivalents of HSA21, we can quickly and easily observe the *in vivo* effects of post-embryonic down-regulation of any target genes (Timmons *et al.* 2001). This allows us to make conclusions regarding the function of these genes. This information can be extrapolated to humans and may lead to inferences about the genes' contributions to the phenotypes of people with DS.

Methods

Generation of RNA-expressing bacterial strains. We obtained 192 RNA-expressing bacterial strains (listed in Appendix) from Geneservice (Nottingham, UK) that correspond to genes on HSA21. Most of the strains were publically available. An additional 33 strains were custom ordered through Geneservice specifically for this project and have not been studied previously. However, 21 (Table 1) of the cultures generated by Geneservice were not able to grow. Because Geneservice was unsuccessful, it is possible that there is a biological reason that bacterial strains expressing these genes

are difficult to successfully generate. Nonetheless, it is important that we examine as many genes as possible, so we attempted to generate these strains ourselves.

We started by examining the gene sequences as they are curated on Wormbase (Release WS203) and designing primers to cover approximately 300-500 bp of gene exon sequence. Using a standard restriction digest and ligation cloning protocol (Fraser *et al.* 2000), we inserted the gene sequence into L4440 plasmids. This plasmid was used to generate the RNAi by feeding strains in both the original study (Timmons *et al.* 2001) and in the RNAi library obtained from Geneservice (Nottingham, UK). Appropriate insertion was confirmed by PCR. Correctly cloned plasmids were transformed into HT115 strain of *E. coli*, again following the established protocol for generation of RNA-expressing bacterial strains for RNAi in *C. elegans* (Fraser *et al*. 2000).

RNA interference. Our RNAi library, compiled by Geneservice (Nottingham, UK), consisted of *E. coli* (strain HT115) transformed with a small portion of an HSA21 gene equivalent cloned into L4440 plasmid, as described above. Neurons in *C. elegans* are known to be resistant to RNAi treatment by feeding (Timmons *et al.*, 2001). Therefore, we used a neuronal RNAi-sensitive *C. elegans* strain, *nre-1(hd20);lin-15b(hd126)* (*BZ1272*), because we were most interested in the neuronal and neuromuscular effects of RNAi treatment (Schmitz *et al.* 2007).

The procedure for RNA-interference is straightforward and takes less than two weeks to complete an assay (outlined in Figure 2). Because minimal active time is required, many assays can be run in parallel. First, RNA-expressing bacteria were cultured overnight at 37°C with shaking in LB broth + ampicilin (to control for

contamination of liquid cultures). The next day, ~500 μL of liquid culture was grown on standard nematode growth media (NGM) plates including 1mM isopropyl ß-D-1-thiogalactopyranoside (IPTG) to induce expression of exogenous RNA by the T7 promoter. Once a bacterial lawn has sufficiently grown, RNAi-sensitive worms were quickly bleached in a 2:1 mixture of bleach and 1 M NaOH to remove any bacteria and kill all post-embryonic worms. A single dead, gravid hermaphrodite containing approximately 8 eggs was picked to each plate. The eggs inside the dead adult were allowed to hatch and grow over the next 8 days at room temperature, with observation on days 1, 2, 4, 6, and 8. This time period covers two generations. The first generation experiences purely post-embryonic effects of the RNAi treatment, while the second generation also experiences maternal or pre-embryonic effects. We identified genes that elicited general phenotypes when knocked down, and also phenotypes known to be involved with neurological and/or neuromuscular functioning, and thus may have an important role in the neurological phenotypes of people with DS.

To examine the effects of the RNAi treatment, we watched for 36 different phenotypes as detailed in Table 2. We were able to observe each phenotype through a simple dissecting microscope in minutes, allowing the screening of thousands of worms during this procedure. The phenotypes on our list can be broken down into two general groups: those with a morphological basis and those often associated with neurological or neuromuscular function. We consider phenotypes resulting in abnormal locomotion or pharyngeal pumping as those with a 'neurological or neuromuscular basis'. Because the worm's nervous system is so compact (with only 302 neurons), a dysfunction in any one

neuron often manifests as a gross phenotype such as abnormal movement. For example, ablation of AVA and AVD neurons prevents the worm from performing reversals in response to probing with a wire, while ablation of dopaminergic neurons will prevent the worm from performing its usual search pattern when looking for food (reviewed by deBono and Maricq 2005). Phenotypes usually associated with neurological components are in bold in Table 2.

The phenotypes we studied are mostly those in common use throughout the worm community, but several were defined specifically for our study. For example, we defined five phenotypes that can be observed after probing the worm on the head with a platinum wire. Usually, a wild-type worm will reverse, propagating 2 to 4 full body bends down the length of its body before going forward again. However, mutant worms will sometimes propagate more body bends (Ere), fewer body bends (Pre), move more slowly (Sre), contract all its body muscles at once (Shr) or become momentarily paralyzed (Fai). We believe it is unlikely that these phenotypes will arise from defects other than those with a neurological or neuromuscular basis.

Results

Generation of RNA-expressing bacterial strains. Despite repeated efforts to generate the missing bacterial strains, we were unsuccessful. Because both we and the Ahringer group (Cambridge) were unable to generate these clones, we suspect that there is something unknown about the genes in question that prevents their successful expression in *E. coli*. Alternatively, it is possible that the genes may share sequence

similarity with the L4440 plasmid, causing secondary structures to form between the gene insert and the plasmid during the cloning process.

RNA interference. More than half of the 170 RNAi treatments tested resulted in an observable neuromuscular or neurological phenotype (Figure 3 and Appendix). Of the 170 genes tested, 64% showed locomotor phenotypes only, while only 2% of treatments elicited exclusively pharyngeal phenotypes. A large portion of treatments (14%) elicited both pharyngeal and locomotor phenotypes simultaneously.

Discussion

We have assayed the *in vivo* function of the great majority of HSA21 gene orthologs identified in our study. The number of RNAi treatments eliciting neurological phenotypes may seem high upon first glance (80%), but there is evidence that approximately two-thirds of all genes are expressed in neurons (Bargmann 1998). As stated previously, any treatment that elicits abnormal locomotion or pharyngeal pumping is considered to have a putative neurological function.

A more in-depth look at the treatments eliciting neurological effects revealed some interesting results. For example, a large portion of RNAi treatments elicited abnormal phenotypes during reversals (Ere, Fai, Pre, Shr, Sre, and Ure). Some of the genes which elicited these phenotypes, such as worm orthologs of *DYRK1A* (Pre), *DSCR1* (Pre), and *SOD1* (Sre), have not previously been associated with neurological phenotypes. This makes sense because the reversal phenotypes defined for this project have never before been studied, and the putative neurological functions of these genes have gone unexamined in worms until now. Interestingly, overexpression of the human

gene *DYRK1A* has been shown to cause developmental brain defects in partially trisomic mice (Guedj *et al*. 2009).

A second interesting group is the treatments that elicited the shrinking (Shr) phenotype. Shrinking worms contract all muscles when they are tapped on the head with a platinum wire, which makes their bodies appear to shrink. Shrinking suggests these genes are involved in GABAergic signaling. Reduction in GABA signaling due to gene knockdown or mutation removes neurochemical inhibition on body muscles. Thus making the worm more likely to contract its body wall muscles simultaneously, leading to shrinking of the body. Seven RNAi treatments were observed to elicit the shrinker phenotype, orthologs of *STCH, ZNF294, C21orf45, DONSON, HMGN1, PRDM15,* and *TRPM2*. None of these human genes or their *C. elegans* orthologs have been previously implicated in GABA signaling. Future studies in our lab will investigate whether these genes are expressed and/or function specifically in GABAergic neurons.

In addition, we identified 27 RNAi treatments which elicit pharyngeal pumping and morphological abnormalities. As stated before, we used the pharynx as a model for cardiac function due to its molecular and functional similarities to the human heart. Several genes in this group have not previously been identified as being involved in pharyngeal function. Interestingly, orthologs of four genes identified in this screen (*CLIC6, ABCG1, CRYAA,* and *PFK*L) are located in an HSA21 cardiac function susceptibility region (Barlow *et al*. 2001). This finding together with the data gathered from the rest of the RNAi screen, suggests that our technique of gene knockdown through

RNAi is a valid method not only to confirm gene function in a neurological and neuromuscular context, but also as a method to implicate new genes in these processes.

RNAi is an excellent tool for studying functional genetics in *C. elegans*, but the technique does have some drawbacks. While we try to standardize treatments, RNAi is notorious for producing variable results. For example, an individual worm may eat more or less RNA-expressing bacteria than other worms, exposing itself to more or less RNAi. This can result in a population of worms with a range of phenotypes, from mild to severe. To make sure that we did not discount a gene treatment because the phenotype showed low penetrance due to the variability of RNAi treatment, we counted every instance of abnormal phenotypes. As long as a single worm in a population exhibited a phenotype, that treatment was scored for that phenotype. This, while it may increase the number of false positives, prevents us from missing genes that may be relevant to the phenotypes of DS. Any false positives we obtained can be identified by further RNAi screening and eventual analysis of equivalent mutant worm strains.

Chapter 4: Screen of synaptic function of HSA21 equivalents using aldicarb

Purpose

Synaptic transmission is vital to an organism's neurological and neuromuscular function. One technique that lends itself well to *C. elegans*, but is impractical in other organisms, is the analysis of synaptic transmission through the use of the acetylcholinesterase inhibitor aldicarb (Brenner 1974). The use of this compound, especially in conjunction with an RNAi screen, allows us to quickly identify those gene products which function at the synapse using a simple behavioral assay. The aldicarb-sensitivity assay is a powerful tool, allowing us to quickly and easily identify synaptic functions of the genes of interest.

The aldicarb sensitivity assay takes advantage of the mechanism that exists at the neuromuscular synapse to constrict muscles using the neurotransmitter acetylcholine. In a healthy animal, acetylcholine is released by the presynaptic cholinergic neuron. As the acetylcholine accumulates in the synapse, the postsynaptic muscle is stimulated by neurotransmitter and contracts. The enzyme acetylcholinesterase degrades the acetylcholine, clearing it from the synapse and allowing the muscle to relax (Brenner 1974; Mahoney *et al*. 2006). Aldicarb is an acetylcholinesterase inhibitor, preventing acetylcholine from being degraded within the synapse. Exposure to aldicarb causes acetylcholine build-up in at the synapse, paralyzing the worms over time due to overstimulation and contraction of muscles (Mahoney *et al*. 2006). The amount of exposure required to cause a worm to become paralyzed can be altered in mutant or RNAi-treated animals. For example, if a gene that has been mutated or knocked down

by RNAi is involved in clearing the acetylcholine or reducing the amount of acetylcholine that is initially released into the synapse, the worm will become paralyzed more quickly when exposed to aldicarb (hypersensitive, Figure 4a). Mutations and expression knock down of different genes can cause the worm to become resistant to aldicarb treatment (Figure 4b). Key genes that are involved in the packaging and release of acetylcholine at the synapse have been identified through aldicarb screens (Brenner 1974; Mahoney *et al*. 2006).

Methods

Gene selection. Because the gene selection assay for all 192 HSA21 gene equivalents would have taken too long, we selected and assayed 21 genes based on their putative neurological or neuromuscular function. Genes whose RNAi treatments resulted in locomotion or pharyngeal pumping anomalies in the worm were chosen first. We further narrowed the list by removing all genes whose expression patterns were not reported in the worm, or whose expression pattern did not include neurons, muscles and/or pharynx. Of the genes that met these requirements, 28 genes were selected to be studied in the following procedure (Table 3).

RNAi. Prior to undergoing the aldicarb sensitivity assay, worms were fed with RNA-expressing bacteria, as described in the previous chapter. The RNA-expressing bacteria was grown in liquid culture and seeded onto IPTG-containing NGM plates. *BZ1272* worms were bleached, and a dead, gravid adult was placed on each dry, seeded plate. The worms were allowed to grow on the RNA-expressing bacteria for 8 days before undergoing the aldicarb assay.

Aldicarb sensitivity assay. This assay is based on the procedure published in 2006 by Mahoney *et al.*, that standardized the use of aldicarb sensitivity assays in *C. elegans*. After 8 days of feeding on the RNA-expressing bacteria, worms were picked to unseeded plates containing 0.5 mM aldicarb in NGM. Whenever possible, 25-30 young adult worms were assayed from each treatment. RNAi treatments can have many effects, including deficits in viability and offspring production. In these cases, we picked as many adult worms as had survived. All worms were observed at the start of the assay (time=0) to ensure that they were not dead or paralyzed. The number of worms moved to the aldicarb plate was noted, and the assay began. Every hour for the following 6 hours, the number of paralyzed worms was noted. Paralysis was defined as a worm showing no movement or pharyngeal pumping, spontaneously or after three pokes on the head and tail. After 6 hours, the number of remaining, moving animals was noted. Aldicarb assays on RNAi-treated worms were performed in triplicate and seven times for the control condition where animals were fed bacteria with the L4440 plasmid that does not produce double-stranded RNA.

Results

In control experiments, mutant strains that had been previously been shown to be resistant and hypersensitive to the paralyzing effects of aldicarb responded as expected over the 6-hour treatment period in comparison to a wild-type strain (N2) (Mahoney *et al.* 2006). For example, the diacylglycerol kinase theta mutant (*dgk-1*) was hypersensitive because deletion of this gene causes enhanced release of acetylcholine at synapses (Figure 4a), while the synaptotagmin mutant (*snt-1*) was resistant because loss of

function mutations in the gene result in severely compromised synaptic transmission (Figure 4b). These pilot results indicate that our protocol for the aldicarb assay can correctly identify genetic perturbations that up and down-regulate the *in vivo* function of synaptic machinery.

For our RNAi aldicarb sensitivity assay, we found that the fraction of animals moving decreased over the time as above with aldicarb treatment. Animals fed bacteria containing no double-stranded RNA served as the control. On average, these control animals dropped to 70 % moving after 6 hours treatment (n= 7 assays, Figure 4c).

To identify which genes may positively or negatively regulate synaptic transmission, we compared which RNAi treatments caused animals to become paralyzed, on average, faster or slower than control worms. Due to the variable penetrance of RNAi effects, we only considered RNAi treatments that produced average effects above and below the standard error of the mean for the control animals.

Of the 28 genes targeted via RNAi, 7 produced lethality and/or early developmental arrest phenotypes which precluded obtaining enough individual animals for analysis. For the remaining targeted genes, we found that knockdown of 11 of them caused no change from the control group (gray traces in Figure 4c). Knockdown of 5 genes caused animals to show resistance to aldicarb relative to control suggesting roles for these targeted genes in synaptic function. These included worm gene equivalents to the human genes: *ITSN1*, *BRDWR1*, *C21orf63*, *DSCR1*, and *PIGP*/*DSCR5*. Knockdown of 5 additional genes caused animals to show hypersensitivity to aldicarb relative to control, suggesting roles for these targeted genes in negatively regulating synaptic

function. These included worm gene equivalents to the human genes: *TTC3*, *SFRS15*, *GABPA*, *ANKRD21*/*POTE*, and *NCAM2*.

Discussion

The greatest advantage of the aldicarb-sensitivity assay is that it permits the assignment of synaptic functionality to specific genes based on a simple behavioral screen rather than through extensive electrophysiological analyses. Those genes designated hypersensitive (those which became paralyzed more quickly, and at a higher percentage than wild-type) likely function *in vivo* in removal or prevention of acetylcholine entering the synapse. These worms become paralyzed more quickly than wild-type worms due to more rapid buildup of acetylcholine in the synapse. While some of these genes (*TTC3* and *GABPA*) have been studied for their role in neuronal function, others including *SFRS15* and *ANKRF21/POTE* have not (Berto et al., 2007; Herndon and Fromm, 2008). Alternatively, genes resulting in aldicarb resistance when knocked down are likely involved in the production, packaging or release of acetylcholine into the synapse. Knockdown of these genes reduces the amount of acetylcholine released into the synapse, lengthening the amount of aldicarb exposure required to induce paralysis. Many of the resistant genes identified here were previously indicated as potentially being important in the DS critical region (*ITSN1*, *DSCR1*, and *PIGP*/*DSCR5*) (Belichenko et al., 2009). However, other genes have not been studied at all in the context of neurological dysfunction in DS (*BRDWR1* and *C21orf63*). Genes identified in our aldicarb screen will be studied further with the *C. elegans* system to investigate their cellular and subcellular roles in synaptic transmission.

Chapter 5: Future directions

In the future, we intend to generate bacterial strains expressing double-stranded RNA to target the 21 as yet unrepresented genes. While we were unable to generate these strains, there are alternative methods for strain generation. For example, we would like to try to generate these strains using a different plasmid. L4440 (the plasmid used for the existing RNAi strains) lacks a blue/white screening element, making the screening of transformed strains difficult and time consuming. We could obtain or generate a plasmid with the same elements as L4440, but which includes a blue/white screen element. Second, we suggest that using different or smaller portions of the genes in question could make the generation of clones easier.

Furthermore, we plan to continue the aldicarb sensitivity assay, increasing the numbers of worms tested with each RNAi treatment will help to overcome the inherent variability of the procedure. We also plan on screening the remaining 162 HSA21 genes that have been untested to date. While we attempted to enrich for genes that had a role in neurological or neuromuscular function, it is possible or even likely that there are other HSA21 genes with functions relevant to DS-associated phenotypes.

We will also confirm the results of the RNAi screen through the use of available worm knock-out strains. Only a portion of the HSA21 gene equivalents have been knocked out in worms, but these can be helpful to determine the true validity of our RNAi screen. We will examine the knock-out worms, watching for the 36 phenotypes we used in our RNAi screen and performing aldicarb-sensitivity assays. This will provide alternative evidence for our results from the RNAi-treated worms.

While knockdown studies can tell us a lot about the function of HSA21 genes, DS is a disease of overexpression. Thus, we also need to examine the neurological and neuromuscular affects of overexpression of HSA21 gene equivalents. To do this, we will overexpress HSA21 gene equivalents one at a time or in groups in wild-type worms. Single extra copies of genes of interest can easily be introduced into the genome through the use of MosSCI single-copy insertion techniques (Frøkjaer-Jensen *et al*. 2008). Our lab has already begun this work, with promising results (Griffith and Pierce-Shimomura unpublished data; Crisp and Pierce-Shimomura unpublished data).

Chapter 6: Conclusion

Our research is a step toward understanding the biology of DS and the contribution of HSA21 genes to the phenotypes associated with DS. While previous research has focused on the functions of larger groups of genes (the DS critical region, for example), we are the first to use a systematic approach to study the individual *in vivo* functions of such a large set of HSA21 genes in any animal. Their genetic similarities to humans and ease of use as a neurological model make *C. elegans* a more powerful tool for this kind of study than any other model organism. In addition to mice, for example, cell lines have been suggested as a model for DS, but their inherent aneuploidy negates any use they might have in studying a disease of gene dosage (Cimini and Degrassi, 2005). An alternative approach to control chromosome number would use human embryonic stem cells as a model for the effects of trisomy 21, however, this approach has its own limitations with *in vivo* relevance and ethical constraints (Biancotti *et al.* 2010)

We have used RNAi in *C. elegans* to examine the *in vivo* function of HSA21 gene equivalents. We have determined that 80% of the 170 HSA21 gene orthologs exhibit neurological or neuromuscular phenotypes when knocked down. Also, the aldicarb sensitivity assay in conjunction with RNAi has proven to be a particularly powerful tool. This assay has identified several genes with novel functions at the synapse. Genes that we identified as having neurological function or specific function at the synapse in worms are likely to have vital roles in the neurological or neuromuscular function of people with DS.

DS is a disease of overexpression, not of knockdown. While we have studied only the knockdown of gene expression, we can draw conclusions regarding the function of HSA21 genes in the context of overexpression, paving the way for overexpression studies. Often, knockdown of a gene has an opposite effect to that of overexpression (Südhof 2008). Alternatively, in the case of dominant negative effects, knockdown and overexpression will elicit the same phenotype (Südhof 2008). We have made progress in understanding the function of these genes. Knowing the function of HSA21 genes sets the stage for further studies of gene overexpression, and ultimately a deeper understanding of the neurobiology of DS.

Table 1: **21 Genes unrepresented in the RNAi library.** Twenty-one bacterial strains in the RNAi library express RNA with sequence similarity to *C. elegans* HSA21 gene equivalents. We were unable to generate these clones.

Human Gene	*C. elegans* Gene Equivalent	*C. elegans* Short name
LIPI	*R155.1*	*mboa-6*
AF165138.7	*Y77E11A.16*	
MRAP	*C14A4.15*	*srv-2*
URB1	*T05H4.10*	
OLIG2	*Y105C5B.29*	*hlh-32*
TMEM50B	*Y74C10AL.2*	
SLC5A3	*F52H2.4*	
CLIC6	*Y105E8A.22*	*exc-4*
SETD4	*Y92H12BR.6*	*set-29*
WRB	*Y50D4A.2*	
SH3BGR	*E02A10.2*	*grl-23*
PCP4	*ZK1320.13*	
DSCAM	*Y42H9B.2*	*rig-4*
AP001631.10	*Y49E10.29*	
PTTG1IP	*C37C3.12*	
C21orf70	*C18H7.3*	*col-102*
POFUT2	*K10G9.3*	*pad-2*
PCBP3	*Y119D3B.17*	*pes-4*
COL6A1	*M199.5*	*col-135*
MCM3AP	*JC8.13*	*tag-115*
S100B	*F55C10.1*	*cnb-1*

Table 2: **Phenotypes observed.** We screened for 36 different gross phenotypes when conducting our RNAi screen. * Indicates uncommon phenotypic descriptor designed specifically for our study. Phenotypes usually related to neuromuscular and/or neurological development and/or function noted in **bold**. Other phenotypes were same as described in Fraser *et al.* (2000).

Phenotype	**Description**	**Phenotype**	**Description**	**Phenotype**	**Description**
Adl	Adult Lethal	**Hdl***	Abnormal Headlifting	Rol	Rolling
Bli	Blistered Cuticle	Lon	Long Body	Rum	Ruptured Vulva
Bmd	Body Morphology Defective	Lva	Larval Arrest	Sck	Sick Appearance
Clr	Clear Appearance	Lvl	Larval Lethal	**Shr***	Shrinking
Daf-c	Dauer Formation Constitutive	**Mec**	Mechano-sensory Defective	**Slu**	Sluggish
Dpy	Dumpy	Mlt	Molting Defects	**Sre***	Slow Reversals
Egl	Egg Laying Defective	Muv	Multiple Vulvas	Ste	Sterile
Emb	Embryonic Lethal	**Pmd***	Pharynx Morphology Defect	Stp	Sterile Progeny
Ere*	Extended Reversals	**Pmp***	Pharynx Pump Defect	**Unc**	Uncoordinated
Fai*	Fainter	**Pre***	Premature Reversals	**Unm***	Unmotivated
Gro	Growth Defective	**Prz**	Paralyzed	**Ure***	Uncoordinated Reversals
Him	High Incidence of Males	Pvl	Protruding Vulva	**Vac**	Vacuole/Cell degeneration

Table 3: **Genes selected for screening with the aldicarb sensitivity assay.** Thirty genes were selected for screening with the aldicarb sensitivity assay. This list was enriched for genes likely involved in neurological or neuromuscular function. * indicates strains that did not grow, and were not assayed for aldicarb sensitivity.

Human Gene	*C. elegans* Gene Equivalent	*C. elegans* Short name
TPTE	*T07A9.6*	*daf-18*
ANKRD21	*T28D6.4*	
RBM11	*C08B11.5**	*sap-49*
NCAM2	*F15G9.4*	*him-4*
NCAM2	*ZK377.2*	*sax-3*
MRPL39	*C47D12.6**	*trs-1*
GABPA	*T08H4.3*	*ast-1*
GABPA	*C42D8.4*	*ets-5*
GABPA	*C37F5.1*	*lin-1*
APP	*C42D8.8**	*apl-1*
ADAMTS1	*F25H8.3**	*gon-1*
ADAMTS5	*C37C3.6**	*mig-6*
SFRS15	*D1007.7*	*nrd-1*
HUNK	*T01C8.1*	*aak-2*
C21orf63	*F32A7.3*	*eva-1*
ITSN1	*Y116A8C.36*	*itsn-1*
DSCR1	*F54E7.7*	*rcn-1*
CLIC6	*F26H11.5*	*exl-1*
RUNX1	*B0414.2*	*rnt-1*
DOPEY2	*Y18D10A.13**	*pad-1*
PIGP	*Y48E1B.2*	
TTC3	*R09E12.3*	*sti-1*
DYRK1A	*F49E11.1*	*mbk-2*
BRWD1	*F55B12.3*	*sel-10*
MX2	*C02C6.1*	*dyn-1*
RIPK4	*B0350.2*	*unc-44*
ABCG1	*F19B6.4*	*wht-5*
TSGA2	*T22C1.7*	*jph-1*
SUMO3	*K12C11.2**	*smo-1*

Figure 1: ***C. elegans* equivalents of HSA21 protein coding genes**. The numbers represent human genes which show sequence similarity to a *C. elegans* gene. Including keratin genes, 67% of all HSA21 protein-coding genes are represented in the *C. elegans* genome. Excluding keratin genes, 85% of HSA21 protein-coding genes are represented.

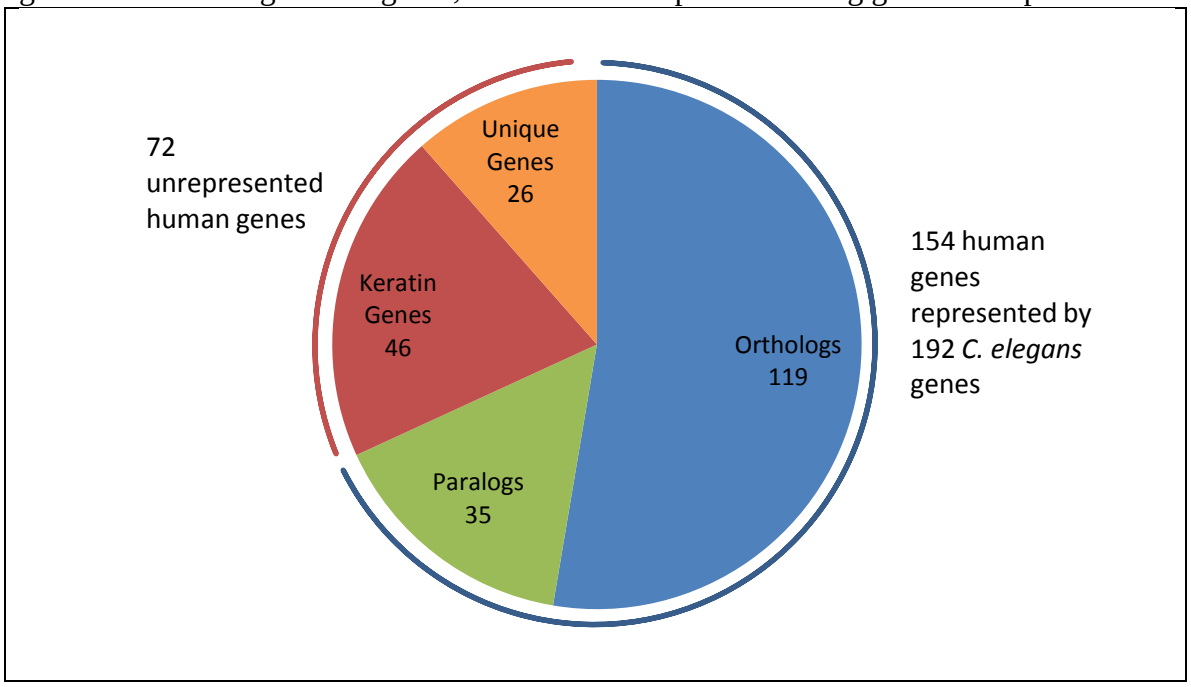


Figure 2: **Outline of RNAi procedure.** Negative control: HT115 *E. coli* containing L4440: no phenotype. Positive control: *apl-1*, phenotypes: sterile, larval arrest. RNAi treatments were assigned random codes to enable unbiased testing.

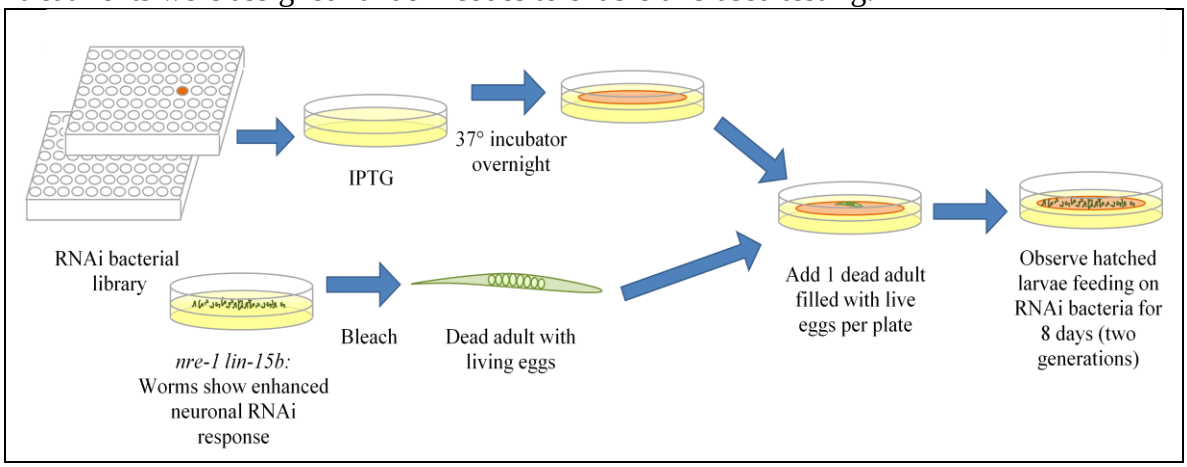


Figure 3a: **Results of RNAi screen**. Percentage of total worms displaying neurological or neuromuscular phenotypes when treated with RNAi.

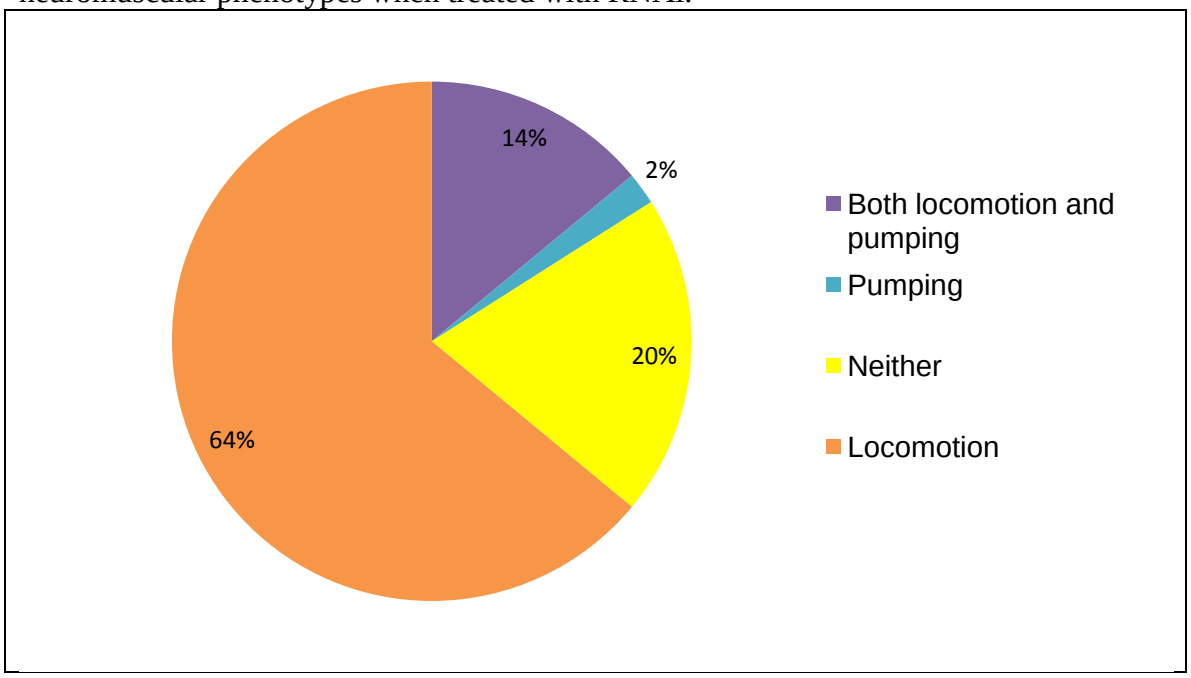


Figure 3b. **Percentage of worms displaying each phenotype.** An individual worm may exhibit more than one of the following phenotypes.

Locomotion (%)				Pharyngeal Pumping (%)		Other Phenotypes (%)					
Ere	4.7	Shr	4.7	Pmd	1.1	Adl	1.7	Emb	18.2	Muv	1.1
Fai	4.7	Slu	2.9	Pmp	15.8	Bli	1.7	Gro	8.8	Pvl	45.8
Hdl	4.1	Sre	13.5			Bmd	50.5	Him	0.0	Rol	2.3
Mec	4.7	Unc	15.2			Clr	5.2	Lon	0.0	Rup	22.3
Pre	41.1	Unm	9.4			Daf-c	2.3	Lva	2.9	Sck	0.0
Prz	4.1	Ure	10.0			Dpy	5.2	Lvl	0.5	Ste	14.7
						Egl	50.0	Mlt	2.9	Stp	1.1

Figure 4a. **Hypersensitive mutant example.** Aldicarb hypersensitive (Hic) mutant, *dgk-1*, compared to wild-type in an aldicarb sensitivity assay.

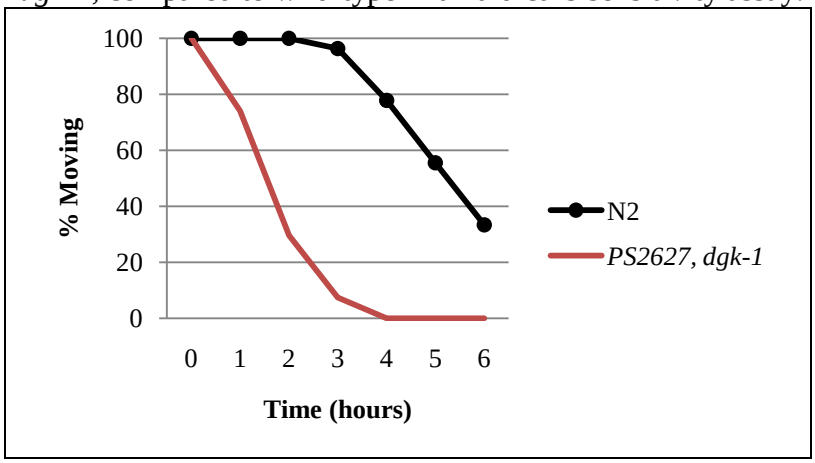


Figure 4b. **Resistant mutant example.** Aldicarb resistant (Ric) mutant, *snt-1*, compared to wild-type in an aldicarb sensitivity assay.

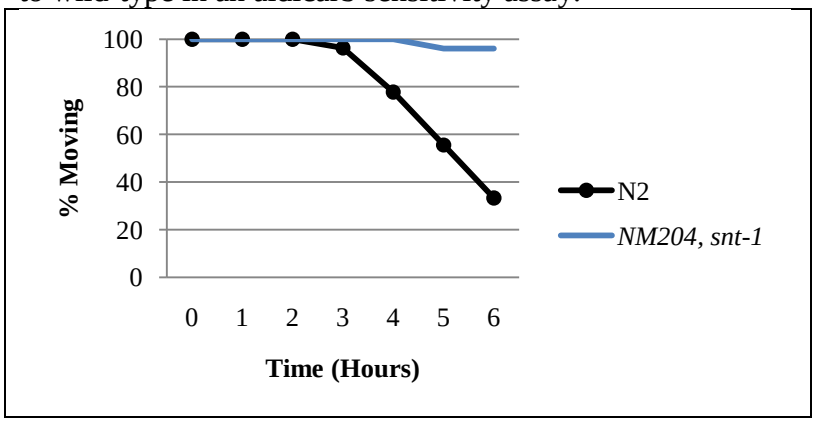
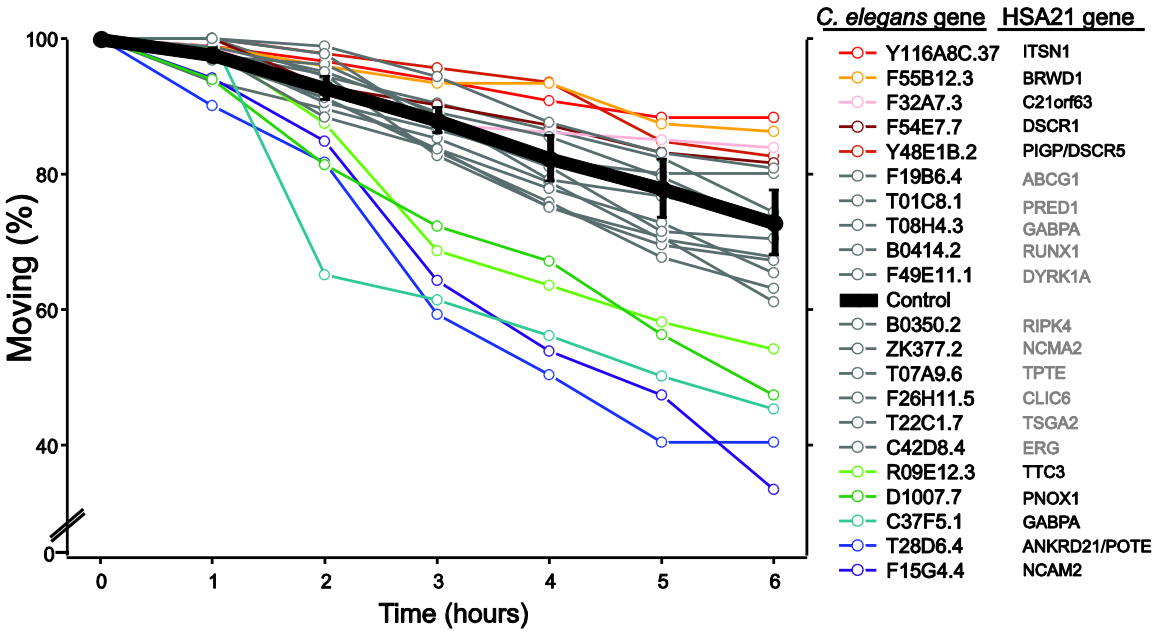


Figure 4c. **Plot of average percentage of animals moving per assay over time course of aldicarb exposure.** Each assay included 20 or more individuals. Control worms were not subject to gene-specific RNAi treatment (black line). Error bars represent s. e. m RNAi treatments that were not significantly different from control are represented in gray, while those that produced significant hypersensitivity or resistance to aldicarb treatment are represented in cool or warm colors, respectively. Error bars for these later groups do not overlap with those from the control group and are not plotted for clarity. Legend names with *C. elegans* and human equivalent genes are listed on the right.



Appendix: **RNAi screen results**. Check marks indicate publically available mutant strains (Red=*C. elegans* Genetic Center, Minnesota, USA; Blue= National Bioresource Project, Japan). Genes are listed in the order they are arranged on HSA21. Genes without a BlastP E-value are paralogs.

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
K04F10.4	*bli-4*	*IGHV1OR1 5-9*			Stez		Unr, Prz, Pre	Pmp	Bmd, Rup
T07A9.6	*daf-18*	*TPTE*	√	7 E-31	Emb		Unc, Pre		Pvl, Bmd
T28D6.4		*ANKRD21*	√	1 E-16			Pre		Pvl, Bmd
C08B11.5	*sap-49*	*RBM11*		1 E-13	Ste	Gro	Unc, Unr	Pmp	Dau, Bmd
Y37D8A.21		*RBM11*	√	1 E-05					Pvl, Egl
F54C9.2	*stc-1*	*STCH*	√	2 E-97	Ste		Shr, Pre, Sre	Pmp	Pvl, Bmd, Rup
Y55B1BR.3		*SAMSN1*			Emb		Unc, Pre		Egl, Bmd, Rup
Y40C5A.3		*NRIP1*					Pre		Egl, Vac
R10E11.3	*usp-46*	*USP25*	√	1 E-04			Ure		Egl
C18A11.7	*dim-1*	*CXADR*	√	0.038	Emb, Lvl	Gro	Pre		Dau, Bmd, Vac,Rup
C01G8.9	*let-526*	*BTG3*			Emb			Pmp	Pvl, Bmd, Vac
K01G5.1	*rnf-113*	*C21orf91*	√		Ste		Unr		Pvl, Bmd, Clr, Rup
B0218.6	*clec-51*	*CHODL*		3 E-08			Sre		Pvl, Rup
ZK546.15	*try-1*	*PRSS7*	√	4 E-37	Emb		Slu, Pre		Pvl, Bmd
F15G9.4	*him-4*	*NCAM2*		3 E-36	Ste		Unc		Rup
ZK377.2	*sax-3*	*NCAM2*		3 E-34	Stp		Unc, Pre, Prz		Pvl, Bmd, Rup
C47D12.6	*trs-1*	*MRPL39*		8 E-12	Ste		Slu		Bmd
Y46H3A.7		*MRPL39*		5 E-08	Emb		Unc, Pre	Pmp	Pvl, Egl
F41D9.3	*wrk-1*	*JAM2*	√√	0.01	Emb				Rup
C09D1.1	*unc-89*	*JAM2*	√√	3 E-10		Gro			Bli
T08H4.3	*ast-1*	*GABPA*	√	2 E-29			Pre		Egl

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
C42D8.4	*ets-5*	*GABPA*	√	1 E-29	Emb		Ure		Clr
C37F5.1	*lin-1*	*GABPA*		2 E-23			Sre		Muv, Pvl, Bmd
C42D8.8	*apl-1*	*APP*	√√	9 E-30	Ste	Lva	Ure		Bmd
ZK1010.7	*col-97*	*CYYR1*			Emb				Pvl
F25H8.3	*gon-1*	*ADAMTS1*	√	5E-159	Ste		Ure		Pvl, Bmd
C37C3.6	*mig-6*	*ADAMTS5*	√	6 E-41	Ste		Sre, Unr	Pmp	Pvl, Rup
C33C12.9		*HEMK2*	√	1 E-38			Pre	Pmp	Egl, Rup
Y54E10A.11		*ZNF294*		6 E-43			Shr, Pre, Unr, Prz		Pvl, Egl, Bmd, Rup
K09C4.8	*sul-1*	*C21orf6*	√	7.3	Emb				
F07A11.4		*USP16*	√√	1 E-16					Pvl, Egl, Bmd, Rup
Y55F3AR.3	*cct-8*	*CCT8*		1 E-153	Ste, Adl	Lva			
C33D9.8		*C21orf7*							Pvl, Bmd
T19E7.2	*skn-1*	*BACH1*	√√	7 E-05	Emb		Unr		Pvl, Bmd
B0280.12	*glr-2*	*GRIK1*	√√	1 E-110			Pre	Pmp	Pvl, Vac
C06E1.4	*glr-1*	*GRIK1*		1 E-125			Sre, Ure		Egl, Bmd
ZC196.7	*glr-5*	*GRIK1*	√	1 E-132			Pre		Egl
Y51A2D.5	*hmit-1.2*	*CLDN17*			Emb			Pmp	Bmd
K04E7.2	*pept-1*	*CLDN8*	√√	0.065					Dau, Pvl, Egl, Bmd
C15F1.7	*sod-1*	*SOD1*	√	8 E-41			Sre		Egl, Bmd
ZK430.3	*sod-5*	*SOD1*	√	1 E-35			Unc		Egl
D1007.7	*nrd-1*	*SFRS15*	√	1 E-14	Stp		Unm, Sre		Bmd
T01C8.1	*aak-2*	*HUNK*	√	8 E-54			Unc, Pre	Pmp	Egl
H39E23.1	*par-1*	*HUNK*	√√	4 E-52	Emb				Pvl, Bmd, Rup

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
F15A2.6	*sad-1*	*HUNK*		2 E-45	Ste, Adl		Pre, Ere		Pvl, Bmd
K02B12.5		*C21orf45*		3.1	Ste		Shr		Egl
F32A7.3	*eva-1*	*C21orf63*	√√	2 E-22			Ere		
B0457.1	*lat-1*	*C21orf63*	√	6 E-07			Sre		Bmd, Clr
F28D9.1	*rsr-1*	*TCP10L*	√√				Pre		Bmd, Rup
F41H10.4		*C21orf59*					Sre, Pre, Unc, Rol		Pvl, Egl
JC8.10	*unc-26*	*SYNJ1*		1 E-122					Egl
F43G9.12		*C21orf66*	√	2 E-09					Pvl, Egl, Bmd, Clr
C06G1.2		*C21orf49*					Sre	Pmp	Pvl, Egl
M142.2	*cut-6*	*C21orf62*	√	0.4			Ure, Rol		Bmd
F38C2.2	*hlh-17*	*OLIG2*	√√	3 E-19					Bmd
C04E6.4		*C21orf54*		0.94		Gro	Sre	Pmp	
Y48A6B.1		*IFNAR2*		8.6			Unc, Ere, Pre		Bmd
C44C10.9		*IL10RB*					Pre, Hdl		Egl
H19M22.2	*let-805*	*IL10RB*		7.4			Unc		Bmd
C09D8.1	*ptp-3*	*IFNAR1*	√√	2 E-05	Emb		Unc, Ure		Egl, Bmd, Rup
C56C10.13	*dnj-8*	*C21orf55*		0.001			Pre		Egl
F38B6.4		*GART*		1 E-28					Pvl, Dpy
D1037.1		*SON*	√	4 E-40			Unm, Unc		Pvl, Egl
C24H12.5		*DONSON*	√	7 E-12			Shr		Bmd, Vac
D2063.1		*CRYZL1*		0.024			Fai		Pvl, Egl
Y116A8C.36	*itsn-1*	*ITSN1*	√√	1 E-45			Sre, Unr		Egl
F27C1.7	*atp-3*	*ATP5O*		9 E-35	Ste	Lva	Unm	Pmp	

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
R12E2.12		*MRPS6*		3 E-06	Ste	Gro	Unm	Pmp	Bmd
C29F5.4	*mps-1*	*KCNE2*	√	0.41			Ere, Sre	Pmp	Mlt, Bmd
T28D9.7		*AP000322.54*	√			Gro	Hdl		Bmd, Clr
Y57A10B.1		*AP000322.53*		0.077				Pmp	Egl
F54E7.7	*rcn-1*	*DSCR1*	√	2 E-32			Pre		Pvl, Bmd
F26H11.5	*exl-1*	*CLIC6*	√√	2 E-12			Ure		Pvl, Bmd, Clr
B0414.2	*rnt-1*	*RUNX1*	√√	2 E-25			Sre, Pre		Egl, Bmd, Clr
F20G2.1		*CBR1*	√	1 E-11		Gro	Slu, Ere		Bmd
C15H11.4	*dhs-22*	*CBR1*		5 E-13	Ste		Prz, Pre, Bme	Pmp Pmd	Egl
Y18D10A.13	*pad-1*	*DOPEY2*		9 E-61			Ure, Pre, Rol	Pmp	Pvl, Bmd
ZC155.3	*morc-1*	*MORC3*		9 E-23			Unm		Bmd, Rup
Y71G12B.1		*CHAF1B*		3 E-58			Ere, Prz		Bmd, Dpy, Rup
D1069.1		*CLDN14*					Unc, Pre		Bmd
F38A6.3	*hif-1*	*SIM2*	√	2 E-22			Unm, Hdl		Bmd
F13H8.10	*bpl-1*	*HLCS*	√	5 E-54			Unc, Pre		Mlt, Pvl, Egl
F14F7.1	*col-98*	*DSCR6*					Unc, Ure		Pvl, Bmd
Y48E1B.2		*PIGP*		2 E-11	Emb		Slu		Bmd
C07G1.1	*try-2*	*TMPRSS3*	√	3 E-33			Unm, Pre		Pvl, Egl
R09E12.3		*TTC3*	√	1 E-06		Gro	Unm, Ure		Bmd
T20D3.7	*vps-26*	*DSCR3*	√	8 E-11	Ste	Gro			Bmd, Dpy
T04C10.1	*mbk-1*	*DYRK1A*	√	1 E-141			Pre	Pmp	Mlt, Clr, Rup, Bli
F20B6.8	*hpk-1*	*DYRK1A*		9 E-57	Emb		Pre		Egl
F49E11.1	*mbk-2*	*DYRK1A*	√	4 E-80			Unc, Sre, Ere, Hdl		Pvl, Egl

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
M02A10.2	*irk-2*	*KCNJ6*		2 E-90			Sre		Egl, Dpy
R03E9.4	*irk-1*	*KCNJ6*		1 E-85					Pvl, Egl
K04G11.5	*irk-3*	*KCNJ6*		2 E-54					Pvl, Egl
F48B9.1		*DSCR4*		0.65	Emb		Pre		Egl, Vac
R06C1.3	*wve-1*	*DSCR8*		6.2	Emb			Pmp	Pvl, Rup
C01G8.2	*cln-3.2*	*PSMG1*	√	5.5			Shr		Mlt, Pvl, Egl, Bmd
F55B12.3	*sel-10*	*BRWD1*	√	6 E-17			Pre		Pvl, Egl, Bmd
H39E23.3		*HMGN1*							Egl, Rup
F58G4.1	*myo-5*	*C21orf13*	√	0.002			Pre, Prz		Pvl, Bmd
Y105E8A.1	*yrs-2*	*SH3BGR*	√	2 E-09	Emb		Pre	Pmd, Pmp	Bmd, Vac, Rup
B0024.15		*B3GALT5*		2 E-16			Pre		Egl, Dpy, Rup
T12G3.8	*bre-5*	*B3GALT5*		1 E-18		Gro	Fai		Pvl, Egl
T19B4.7	*unc-40*	*DSCAM*		9 E-42					
R12H7.2	*asp-4*	*BACE2*	√	4 E-19			Ure		Egl, Bmd
Y39B6A.20	*asp-1*	*BACE2*	√	2 E-13			Pre, Hdl		Egl, Bmd, Rup
H22K11.1	*asp-3*	*BACE2*		3 E-21			Pre		Pvl, Egl
M70.4		*FAM3B*		2 E-17			Ure		Bmd
C02C6.1	*dyn-1*	*MX2*	√	8 E-15	Ste	Lva	Unc		Bmd
C43G2.5	*try-3*	*TMPRSS2*		2 E-15					Egl, Dpy
B0350.2	*unc-44*	*RIPK4*	√	6 E-45	Emb		Pre, Shr		Pvl, Egl, Bmd, Rup
F28F9.1	*zag-1*	*PRDM15*	√√	1 E-07			Fai		Egl, Pvl
Y38H8A.5		*PRDM15*	√	1 E-14		Gro	Pre		Pvl, Egl
R11G1.6		*C2DC2*		5 E-04					Mlt, Pvl, Bmd

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
R11E3.6	*eor-1*	*ZNF295*	√√	9 E-08					Pvl
F41F3.4	*col-139*	*UMODL1*					Rol		Egl, Bmd, Rup
Y42G9A.6		*ABCG1*	√	5 E-66			Pre, Ure		Pvl, Egl, Clr
F19B6.4		*ABCG1*	√	1 E-75		Gro	Pre	Pmp	Pvl, Egl, Rup
C05D10.3	*wht-1*	*ABCG1*	√	2 E-81	Emb	Gro	Unm, Pre		Egl
F02E11.1	*wht-4*	*ABCG1*	√	1 E-69			Pre		
T19D12.1		*TFF2*			Emb		Pre		Pvl
T07F12.1		*UBASH3A*		8 E-45	Emb		Unm, Pre, Ere		Pvl, Egl, Bmd
T22C1.7	*jph-1*	*TSGA2*	√	6 E-11			Pre		Pvl, Egl, Vac
T11G6.2		*SLC37A1*		3 E-62	Emb				Pvl, Bmd
T04D3.3	*pde-1*	*PDE9A*	√√	3 E-37			Unc, Pre		Pvl, Egl, Bmd, Dpy
Y102E9.2		*AC127383.1*							Egl, Bmd
C25A1.10	*dao-5*	*NDUFV3*	√				Pre	Pmp	
T28F12.2	*unc-62*	*PKNOX1*	√	5 E-24	Ste				Pvl, Egl, Rup, Bmd
ZC373.1		*CBS*		9 E-94	Emb				Egl, Dpy
F54A3.4		*CBS*	√	5 E-92			Pre		Egl, Vac
Y116A8C.35	*uaf-2*	*U2AF1*		1 E-68	Ste	Lva	Pre, Ure		Bmd
T22A3.2	*hsp-12.1*	*CRYAA*	√	2 E-12			Sre, Pre		Pvl, Egl
C14B9.1	*hsp-12.2*	*CRYAA*	√	3 E-14	Emb		Unc, Pre	Pmp	Pvl, Egl, Bmd
F38E11.2	*hsp-12.6*	*CRYAA*	√	1 E-10			Rev		Pvl, Egl
F38E11.1	*hsp-12.3*	*CRYAA*	√	6 E-09					Pvl, Egl
F58H12.1		*SNF1LK*	√	2 E-74			Sre		Egl, Rup
ZK1055.1	*hcp-1*	*HSF2BP*		2.5	Emb				Pvl

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
C47E12.7		*KIAA0179*		7 E-16	Ste	Gro	Unc, Pre, Ure Prz		Pvl, Bmd
F57C9.1		*PDXK*		1 E-54			Sre		Pvl, Bmd
C49A9.4		*CSTB*		0.065			Sre		Egl, Bmd
F55A11.5	*bus-18*	*AGPAT3*	√	3 E-14			Pre		Pvl, Egl, Bmd
Y71G12A.2		*TRAPPC10*		8 E-18			Hdl		
F55F8.3		*PWP2H*	√	0	Ste		Unc, Ure, Pre	Pmp	Muv, Pvl, Bmd
T28C6.1	*grsp-2*	*DNMT3L*	√		Emb				Vac, Pvl, Egl, Bmd
C50F4.2		*PFKL*		1 E-90			Slu, Pre, Sre		Pvl, Egl, Bmd
Y71H10A.1		*PFKL*		1 E-174	Emb		Unm, Pre, Hdl		Egl
F09G8.5		*C21orf2*		3 E-30			Shr		Pvl, Egl, Bmd
F54D1.5	*gtl-2*	*TRPM2*	√	1 E-91	Emb		Pre		Egl, Bmd
T01H8.5	*gon-2*	*TRPM2*	√√	2 E-76			Pre		Egl, Bmd, Rup
C05C12.3	*gtl-1*	*TRPM2*	√√	6 E-74			Pre		Pvl, Bmd, Rup
F40E10.4	*slt-1*	*LRRC3*	√	4 E-12					Egl
T18H9.7	*tag-232*	*C21orf29*		0.83			Pre		Pvl, Egl, Bmd
Y87G2A.9	*ubc-14*	*UBE2G2*		9 E-71	Ste		Unc, Sre, Pre		Bmd, Rup
K12C11.2	*smo-1*	*SUMO3*	√	4 E-18	Ste		Unc, Pre	Pmp	Pvl, Bmd
ZK1058.2	*pat-3*	*ITGB2*		1 E-137	Ste, Adl		Unr		Dau, Vac
T20H4.4	*adr-2*	*ADARB1*	√√	1E-46			Fai		
C36B1.1	*cle-1*	*COL18A1*	√√	2 E-34			Unm, Pre, Sre		Bmd
C06H2.4	*folt-1*	*SLC19A1*	√	7 E-49					Pvl, Egl, Rup
F01G12.5	*let-2*	*COL6A1*		1 E-14			Fai		
K04H4.1	*emb-9*	*COL6A2*		4 E-09			Fai, Pre		Pvl, Rup, Bmd

Predicted Gene	*C. elegans* locus	Human gene	Mutant available	BlastP E-value	RNAi phenotype				
					Nonv	Gro	Vpep		
							Neuromuscular		Other
							Loco-motion	Feed-ing	
F29C4.8	*col-99*	*COL6A2*	√	0.009			Pre, Fai, Shr		Pvl, Egl
C50F7.2	*clx-1*	*FTCD*			Emb				Egl, Dpy
W03D2.1	*pqn-75*	*FTCD*							Egl, Bmd
C15A11.5	*col-7*	*C21orf56*					Unm		Egl
F02D10.1	*col-183*	*C21orf56*				Gro	Pre, Unm		Egl
K06A9.1		*LSS*					Unm, Pre		
Y53H1A.1	*rsy-1*	*C21orf58*					Unm	Pmp	
H40L08.3		*PCNT*		0.1			Fai		Egl
F28B3.1		*DIP2A*	√	1 E-140	Ste		Unc		Pvl, Egl, Rup, Bmd
T07G12.1	*cal-4*	*S100B*	√	0.29					Pvl, Egl
Y113G7B.17		*PRMT2*	√√	2 E-37			Unc, Pre		Pvl, Egl

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